In Situ Radiolysis Electron Spin Resonance Study of the Radical-anions of Substituted Nitroimidazoles and Nitroaromatic Compounds

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Reaction of e_{aq}^-, $(CH_3)_2\dot{C}OH$, $(CH_3)_2C\dot{O}^-$ or $C\dot{O}_2^-$ with a number of substituted nitroimidazoles and nitrobenzenes results in the formation of their radical-anions and in no case were radical-adducts detected. Changes in the e.s.r. spectra of the product species with pH are interpreted in terms of either the protonation of the radical-anion (metronidazole) or the loss of a proton from the radical anion (2-methyl-5-nitroimidazole, 4-nitroimidazole and nitrophenols) and pK values of some of these processes are evaluated. In several cases the dynamics of these acid–base reactions influence the linewidths of the e.s.r. spectra.

The interest in the radiation chemistry of nitroaromatic compounds because of their potential use as radiation sensitizers in radiotherapy [1, 2] has focused attention on the nature and properties of the products which result from the reaction of free radicals with these compounds.[3]

In this paper we have investigated the species formed when the reducing radicals e_{aq}^-, $CO_2^{\dot{-}}$, $(CH_3)_2\dot{C}OH$ and $(CH_3)_2CO^{\dot{-}}$ react with substituted nitroimidazoles related to the sensitizer metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] and have paid particular attention to the acid–base properties of the product radical. The study has been extended to several nitroaromatic compounds of interest.

EXPERIMENTAL

All compounds were of the purest grade available commercially and were used without further purification. Most were obtained from one of the following sources: B.D.H., Aldrich, Hopkin and Williams and Eastman Chemicals. Metronidazole was obtained from May and Baker and 1-(2-hydroxy-3-methoxy-propyl)-2-nitroimidazole (Roche drug Ro-07-0582) was supplied by Prof. G. E. Adams of the Department of Physics, Institute of Cancer Research, Sutton. These compounds were dissolved to form aqueous solutions with concentrations between 10^{-4} and 10^{-2} mol dm^{-3}. The pH of these solutions was adjusted to the desired value by adding sodium hydroxide or perchloric acid solution. For near neutral solutions a phosphate buffer ($\sim 10^{-2}$ mol dm^{-3}) was used. The details of the *in situ* radiolysis steady-state e.s.r. experiments and the method for generating the reducing radicals $(CH_3)_2\dot{C}OH$, $(CH_3)_2C\dot{O}^-$ and $C\dot{O}_2^-$ have been given previously.[4] The hyperfine coupling constants are quoted to ± 0.005 mT and the g-factors to $\pm 0.000\,08$.

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RESULTS AND DISCUSSION

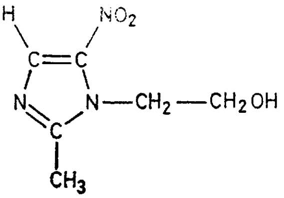
Contrary to an earlier report[5] it was found that for every compound studied the e.s.r. spectra could be interpreted in terms of the reduction of the nitro-compound by the free-radical to the radical-anion, some of which then undergo acid–base reactions (1) or (2)

$$RN(\dot{O})OH \rightleftharpoons RNO_2^{\dot{-}}+H^+ \qquad (1)$$

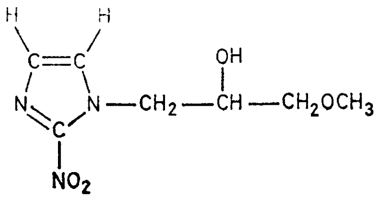
$$HR'NO_2^{\dot{-}} \rightleftharpoons R'NO_2^{2\dot{-}}+H^+. \qquad (2)$$

RADICAL-ANIONS OF NITROIMIDAZOLES

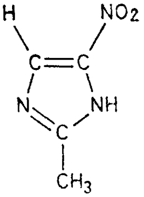
The following compounds were studied:



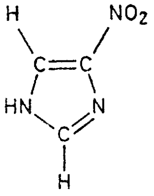
metronidazole



Ro-07-0582



2-methyl-5-nitroimidazole



4-nitroimidazole

(i) METRONIDAZOLE

Solutions containing metronidazole were studied over the pH range 4.9 to 13.3. Reaction with $CO_2^{\dot{-}}$ at pH 5.7 results in an intense spectrum associated with the radical-anion of metronidazole, while at pH 4.9 a weak, poorly resolved spectrum with different e.s.r. parameters was detected (table 1). We have associated this last spectrum with the species resulting from protonation of the anion at the nitro-group.

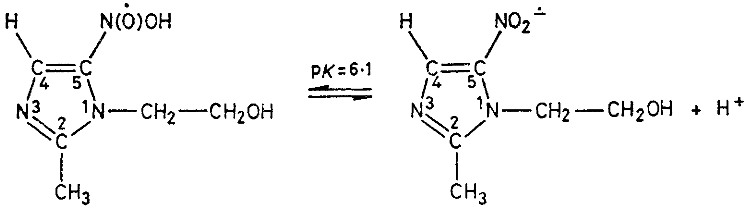
In an earlier publication[5] we incorrectly assigned this spectrum to a radical-adduct between $(CH_3)_2\dot{C}OH$ and metronidazole since it was observed when unbuffered solutions (pH ~ 7) containing 0.1 mol dm^{-3} of propan-2-ol were investigated. However, this was due to the decrease in the pH of the solution resulting from the radiolytic generation of hydrogen ions. On repeating the experiment using phosphate buffered solutions (pH = 7.6) only the radical-anion was observed. These results are in accord with the recently determined[6] value of K_1 (pK_1 = 6.1) of this species. It seems probable that the species formed by reaction in acid solution of metronidazole with $(CH_3)_2\dot{C}OH$, $\dot{C}H_2OH$ and $CO_2^{\dot{-}}$ generated in the $Ti^{3+}+H_2O_2$ system[7] is the protonated species $RN(\dot{O})OH$ and not the radical adduct as suggested by us.[5]

The radical-anion of metronidazole does not appear to be stable in non-polar media. It could not be produced by reduction over a sodium mirror in tetrahydrofuran and also was not observed following reaction with $(CH_3)_2CO^{\dot{-}}$ produced by the photolysis of solutions of acetone in propan-2-ol made alkaline with either sodium hydroxide or triethylamine

$$CH_3COHCH_3 \xrightarrow[h\nu]{(CH_3)_2COH,\ base} (CH_3)_2CO^{\dot{-}}.$$

With the addition of 10 % by volume of water to the system, the intense spectrum of metronidazole radical-anion appeared.

TABLE 1.—E.S.R. PARAMETERS OF THE RADICAL ANION OF METRONIDAZOLE



$a^{N}_{NO_2} = 1.440$ mT	$a^{N}_{NO_2} = 1.565$ mT
$a^{N} = 0.035$ mT	$a^{N}_{1} = a^{N}_{2} = 0.056$ mT
$a^{N} \sim 0.115$ mT*	$a^{H}_{4} = 0.542$ mT
$a^{H}_{CH_2}$ or $a^{H}_{CH_3} \sim 0.115$ mT*	$a^{H}_{CH_3} = 0.229$ mT
	$a^{H}_{CH_2} < 0.02$ mT†
$g \approx 2.0046$	$g = 2.004\,45$

* The spectrum is weak and so these assignments are tentative. There appear to be at least two other non-equivalent proton splittings. † Unresolved structure is evident in some peaks—see figure in ref. (5).

(ii) Ro-07-0582

Only a spectrum ascribable to the radical-anion of Ro-07-0582 was observed over the pH range 5.4 to 13.0. This is consistent with pK_1 for this species being 5.7 as reported by Wardman.[6] The spectrum can be analysed satisfactorily in terms of the parameters: $a^{N}_{NO_2} = 1.410$ mT, $a^{N} = 0.162, 0.098$ mT; $a^{H} = 0.070, 0.042$ mT; $a^{H}_{CH_2} = 0.268$ mT; $g = 2.004\,90$. An earlier reference [2] to this spectrum assigned only the principal nitrogen splitting constant and the g-factor.

(iii) 2-METHYL-5-NITROIMIDAZOLE

This compound is structurally similar to metronidazole. A complete analysis of the e.s.r. spectra of the radical-anion was possible over the pH range 5.0 to 13.8. The hyperfine parameters are summarised in fig. 1. The changes in the coupling constants at the high pH are ascribed to the further ionization of the radical-anion [reaction (2)] presumably by loss of the aminic proton. The dynamics of the processes follow the generalised predictions of Laroff and Fessenden [8] for radicals with a pK in the region 12.5-15.75. The lines are broadened when the pH is ~10 due to the collapse of the doublet splitting associated with the aminic proton as its rate of exchange with the solvent is similar to its hyperfine couplings (expressed in s^{-1}). At pH 11.8 and above the lines have narrowed again as the rate of exchange of the NH proton is sufficiently fast to average its hyperfine coupling to near zero. As the pH approaches the pK of the NH proton all the e.s.r. parameters change as the mean of the couplings of the dissociated and undissociated form weighted by the relative amounts of each species present. The solid lines in fig. 1 have been fitted to the experimental points on this weighted mean basis, assuming $pK_2 = 13.0$ for the radical-anion. Hence the values at pH 15 represent the expected hyperfine coupling parameters for the radical-dianion.

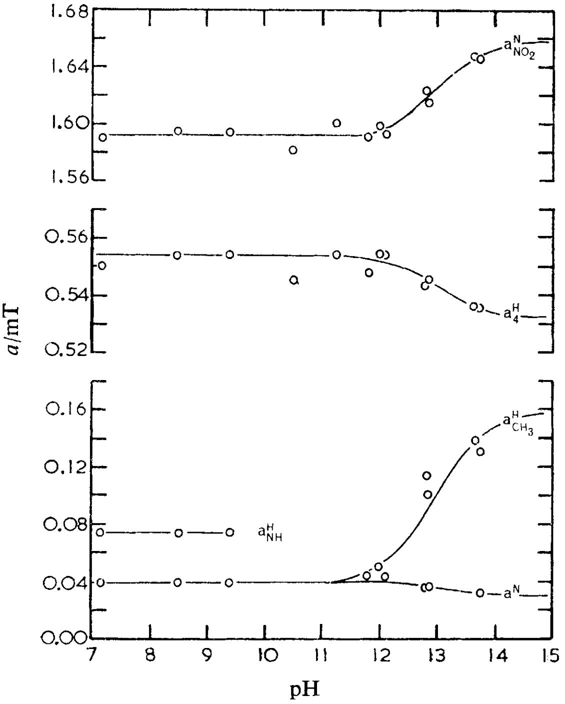


FIG. 1.—Effect of pH on the hyperfine constants obtained for the radical-anion of 2-methyl-5-nitroimidazole. The fitted curves are calculated as described in the text. The *g*-factor for this radical is 2.004 50 at pH 7.2 and 2.004 42 at pH 13.75.

(iv) 4-NITROIMIDAZOLE

The spectra from the radical-anion of 4-nitroimidazole have been observed over the pH range 7.2-13.5. A complete analysis of all the spectra could not be made. However, the coupling constants, $a^N_{NO_2}$ and a^H_5, are easily assigned over the whole pH range and from the variation of these with pH (see fig. 2) it is concluded that $pK_2 = 12.9$ for the radical-anion, the ionization again probably being associated with loss of the aminic proton. As would be expected from the prediction of Laroff and Fessenden,[8] the spectra underwent exchange narrowing in the pH region 10-11.5, due to the exchange of this proton. The remaining coupling constants (a^N_1, a^N_3, a^N_{NH} and a^H_2) could only be assigned above pH 12.4. The spectral lines for these nuclei are shown in fig. 3; all the figures, except for 3[*d*(ii)], show the lowest field group in the spectra; 3[*d*(ii)] is the low field group from the middle set of lines associated with $M^N_1(NO_2) = 0$. For all spectra, except those recorded near pH 11.6, each group was identical (except for linewidth) throughout the spectrum. This was not so for those spectra recorded near pH 11.6 for, whilst the outside groups $M^N_1(NO_2) = \pm 1$ were identical, those associated with $M^N_1(NO_2) = 0$ contained a different number of lines as can be seen in fig. 3[*d*(i) and (ii)]. We cannot suggest an explanation for this. Furthermore the predicted coupling constants for these nuclei at pH 7.4, as assessed from the calculated curves shown in fig. 2, are incompatible with the observed spectrum at this pH [fig. 3(*c*)].

NITROAROMATIC COMPOUNDS

The e.s.r. parameters for a large number of substituted nitrobenzenes were determined and where these duplicated the work of Eiben and Fessenden [9] or Neta and co-workers [10, 11] good agreement was found. All spectra showed the linewidth behaviour associated with nitro-radical-anions in that the intensity of the high field set of lines was reduced relative to the other two.[12] The spectrum of the *m*-dinitrobenzene radical-anion showed, in addition, alternating linewidth as a consequence of the slow libration of the two nitro-groups, one being in the plane of the aromatic ring and the other rotated out of the plane. As with the anion of *p*-dinitrodurene it is the lines associated with $M = M_1^N + M_2^N = 0, \pm 1$ that are broadened.[13]

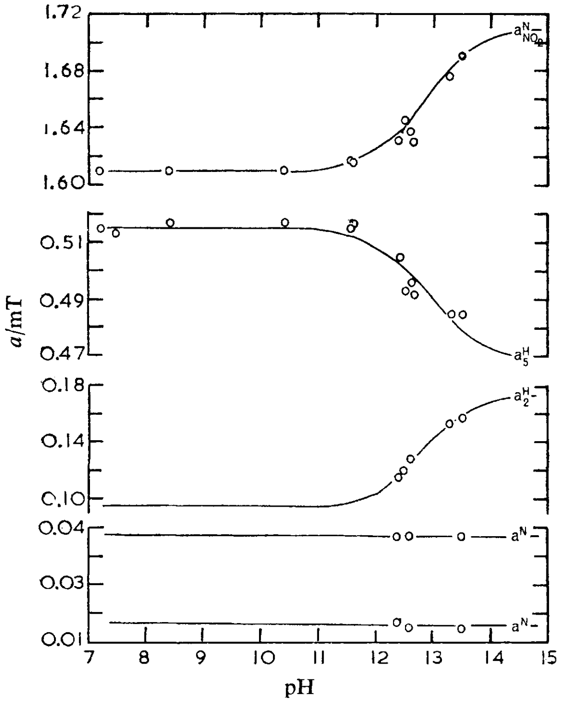


FIG. 2.—Effect of pH on the hyperfine constants obtained for the radical-anion of 4-nitroimidazole. The fitted curves are calculated as described in the text. The *g*-factor for this radical is 2.004 57 at pH 7.2 and 2.004 33 at pH 13.50.

We confirm the report of Eiben and Fessenden [9] that the pK of the *o*-nitrophenol radical-anion obtained from a conductometric pulse radiolysis study [14] is in error. We have also determined pK_2 for the anions of all three nitrophenols

$$NO_2—C_6H_4—OH^{-\bullet} \rightleftharpoons NO_2—C_6H_4—O^{2-\bullet} + H^+. \qquad (3)$$

They are [values from ref. (14) in parenthesis]: *p*-nitrophenol ~9.7 (9.8), *m*-nitrophenol ~9.7 (8.9), and *o*-nitrophenol ~12.7 (9.5). The error in these results is ~ ±0.3 pH units. For the *para* and *meta* species, for which pK_2 < 10, the spectra of the radical-anion and dianion could be detected simultaneously since the rate of exchange of the phenolic proton with the solvent is slow.[8] For the *ortho* species, the spectra of both the anion and the dianion were again observed because hydrogen bonding between the phenolic proton and the nitro-group slows down the rate of exchange of this proton with the solvent which, at pH 12.7, would normally be sufficiently fast to allow only the average spectrum of the two radicals to be detected.[8] The much larger value of $a^{N'}_{NO_2}$ (1.800 mT) for the dianion compared with that of the anion (1.490 mT) arises because hydrogen-bonding between the nitro-group and the hydroxyl group in the anion holds the oxygen atoms of the nitro-group in the plane of the aromatic ring. In the dianion this hydrogen-bonding cannot operate and the nitro-group rotates out of the plane of the aromatic ring to minimise the coulombic forces.

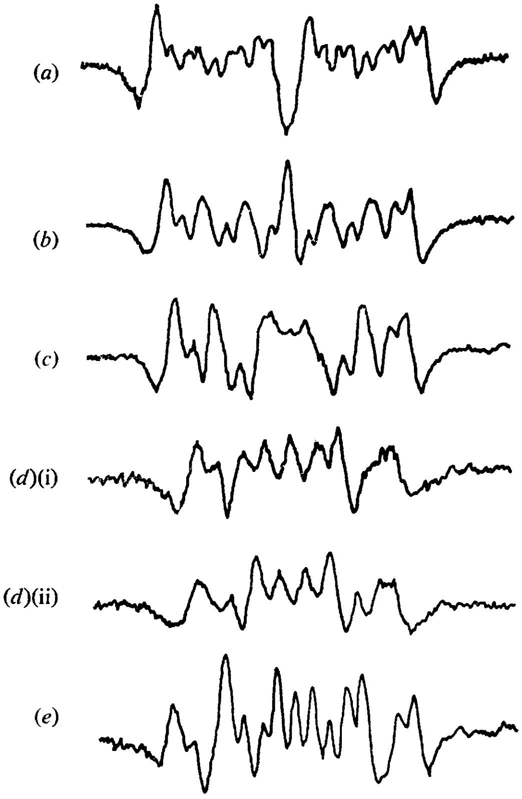
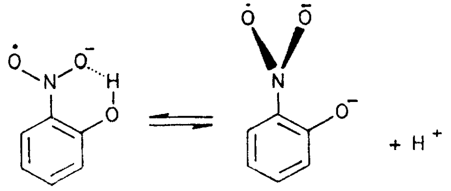
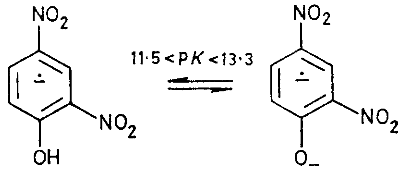


FIG. 3.—The spectral lines associated with coupling constants a_1^N, a_3^N, a_{NH}^H and a_2^H for the radical-anion of 4-nitroimidazole as described in the text and recorded as the second derivative at (*a*) pH 13.5, (*b*) pH 12.6, (*c*) pH 12.4, (*d*) pH 11.6 and (*e*) pH 7.4.



The coupling constants of the ring protons also decrease following the loss of the phenolic proton. These results agree with the prediction of Rieger and Fraenkel [15] for nitro-group rotation in that less spin-density is delocalised onto the ring from the nitro-group as it is rotated out of the plane of the ring. As the value of $a^{N}_{NO_2} \simeq 2.5$-2.6 mT found for nitroalkane radical-anions [16] is associated with the nitrogroup approximately perpendicular to the ring in aqueous solution, (*i.e.* no spin delocalization), the value of $a^{N}_{NO_2} = 1.800$ for the *o*-nitrophenol dianion suggests that rotation of the nitro-group out of the plane of the ring is only partial in this case.

TABLE 2.—E.S.R. PARAMETERS OF THE 2,4-DINITROPHENOL ANIONS*



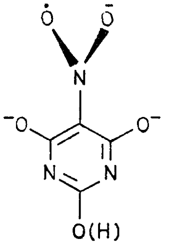
$a^{H}_{6} = 0.081$ mT	$a^{H}_{6} = 0.076$ mT
$a^{H}_{3,5} = 0.169, 0.495$ mT	$a^{H}_{3,5} = 0.162, 0.510$ mT
$a^{N}_{2} = 0.169$ mT	$a^{N}_{2} = 0.222$ mT
$a^{N}_{4} = 1.350$ mT	$a^{N}_{4} = 1.305$ mT
$g = 2.004\,57$	$g = 2.004\,57$

* Ring positions labelled relative to the phenolic group

The study of nitrophenol radical-anions was extended to 2,4-dinitrophenol since by analogy with the nitrophenols it might be possible to estimate which nitro-group has the largest spin density. The e.s.r. parameters observed are given in table 2. The spectrum of the radical-anion was observed at pH 8 and 11.5 and that of the dianion at pH 13.3 and 13.7. No coupling which could be ascribed to the phenolic proton was observed in the anion form and the principal nitrogen splitting constant decreased when the phenolic proton was lost. These results probably indicate that the principal site of electron spin density is the nitro-group *para* to the phenolic group. In the mono-anion the second nitro-group is rotated slightly out of the plane of the ring, whereas in the dianion it lies closer to this plane and hence accepts more of the spin density. This accounts for the decrease in the principal nitrogen coupling and the increase in the small nitrogen coupling in the case of the radical dianion. The larger proton coupling of ~0.5 mT is surprising. However, the average for a^{H}_{3} and $a^{H}_{5} \sim 0.33$ mT is of the order expected when compared with $a^{H}_{2,6}$ for the other nitrobenzene anions.

NITROBARBITURIC ACID

Nitrobarbituric acid was studies as a representative of another class of potential radiation sensitizers. We have produced the anion by electron transfer from $(CH_3)_2CO^{\dot{-}}$ at pH 12.0, 12.1, 12.8 and 13.3. At the two lower pH values, the spectra were weak and had different parameters from those observed at pH 12.8 and 13.3. The parameters of both species are given in table 3. Neither set of coupling constants agrees with those reported by Neta and Greenstock [17] for the nitrobarbituric acid radical-anion at pH 11-12 though the data for the species observed at pH > 12.8 are compatible with a radical-anion (I) with the nitro group rotated so as to be nearly perpendicular to the ring (*cf.* $a^{N}_{NO_2} \approx 2.5$ mT for a number of aliphatic nitro-anions).[16] The very small couplings with the ring nitrogens support the view that there is little delocalization of unpaired spin into the aromatic ring. At lower pH values (12.0-12.2) the three nitrogen hyperfine couplings and the g-factor (see table 3) are plausible for the hydrogen-bonded structure (II) similar to that proposed for the nitrophenol radical anion, but the extremely high proton coupling (1.315 mT) is not reasonable for such a species. This value is in fact typical of a proton in an —NH group and the whole spectrum is not unreasonable for a nitroxide radical $RN(\dot{O})H$ related to the species observed at pH ⩾ 12.8 by loss of O^- and addition of H. We do not, however, suggest that this is the mechanism by which it is formed. Since the spectrum is weak it is more likely to be formed by reduction of a nitroso compound which is itself a product of the reduction of the nitrobarbituric acid in the presence of aqueous isopropanol (*cf.* the photoreduction of nitrobenzene).[18]



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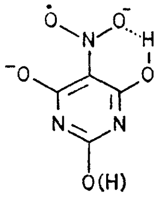

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TABLE 3.—E.S.R. PARAMETERS OF THE SPECIES FORMED BY REDUCTION OF NITROBARBITURIC ACID

pH	$a^{N}_{NO_2}$/mT	a^{H}_{OH}/mT	$a^{N}(2)$/mT	g
12.0, 12.1	1.242	1.315	0.037	2.004 10
12.8, 13.3	2.160	—	0.026	2.005 64

We thank the S.R.C. for financial support.

[1] J. A. Raleigh, J. D. Chapman, J. Borsa, W. Kremers and A. P. Reuvers, *Int. J. Radiation Biol.*, 1973, **23**, 377.

[2] D. W. Whillans, G. E. Adams and P. Neta, *Radiation Res.*, 1975, **62**, 407.

[3] G. E. Adams, *Advances in Radiation Chemistry*, ed. M. Burton and J. L. Magee (Wiley-Interscience, N.Y., 1972), p. 125.

[4] P. B. Ayscough and A. J. Elliot, *J.C.S. Faraday I*, 1976, **72**, 791.

[5] P. B. Ayscough, A. J. Elliot and G. A. Salmon, *Int. J. Radiation Biol.*, 1975, **27**, 603.

[6] P. Wardman, *Int. J. Radiation Biol.*, 1975, **28**, 585.

[7] R. L. Willson, B. C. Gilbert, P. D. R. Marshall and R. O. C. Norman, *Int. J. Radiation Biol.*, 1974, **26**, 427.

[8] G. P. Laroff and R. W. Fessenden, *J. Phys. Chem.*, 1973, **77**, 1283.

[9] K. Eiben and R. W. Fessenden, *J. Phys. Chem.*, 1971, **75**, 1186.

[10] P. Neta and D. Meisel, *J. Phys. Chem.*, 1976, **80**, 519.

[11] P. Neta, M. G. Simic and M. Z. Hoffman, *J. Phys. Chem.*, 1976, **80**, 2018.

[12] F. Millet and J. E. Harriman, *J. Chem. Phys.*, 1966, **44**, 1945.

[13] J. H. Freed and G. K. Fraenkel, *J. Chem. Phys.*, 1962, **37**, 1156.

[14] W. Grünbein and A. Henglein, *Ber. Bunsenges. phys. Chem.*, 1969, **73**, 376.

[15] P. H. Rieger and G. K. Fraenkel, *J. Chem. Phys.*, 1963, **39**, 609.

[16] M. McMillan and R. O. C. Norman, *J. Chem. Soc.* (*B*), 1968, 590.

[17] P. Neta and C. L. Greenstock, *Radiation Res.*, 1973, **54**, 35.

[18] P. B. Ayscough, R. C. Sealy and D. E. Woods, *J. Phys. Chem.*, 1971, **75**, 3454.

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